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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THYMIDYLATE SYNTHASE POLYMORPHISMS FOR USE IN SCREENING FOR CANCER SUSCEPTIBILITY

(57) Abstract: The present invention discloses a novel single nucleotide polymorphism (SNP) in the isolated 5' tandem repeats of the thymidylate synthase (TS) gene and methods for its use. The novel SNP, located in the 12th nucleotide of a 28 bp third tandem repeat (3R) of the TS gene, substitutes a C for a G, and is the variant form of the repeat. Subjects with the wild-type form of 3R have greater transcription of the TS gene than subjects with the variant form. The invention also reveals that a six base pair deletion in the 3' region of TS (-6 bp/1494) indicates mRNA instability and thus reduced production of TS. In diseased tissue, such as cancer, reduced production of TS is beneficial because it prevents the cancerous cells from growing and spreading. Analysis of either polymorphism or both together allows for prediction of a subject's response to chemotherapeutic and anti-cardiovascular disease treatments because both diseases are related to TS levels in a subject.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07H 21/04; C12Q 1/70
US CL : 435/6, 91.2; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.2; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE BIOSIS CAPLUS, Thymidylate shythase, cancer, polymorphism

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database NCBI, Accession Number AF279906, NCBI Entrez database nucleotide sequences, National Center for Biotechnology Information, National Library of Medicine, NIH (Bethesda, MD, USA), 14 Febuary 2001, nucleotides 132-159.	1-10
X	US 6,087,486 A (WEINER et al.) 11 June 2000 (11.06.2000), SEQ ID NO:	1-10
X, P	KAZUYUKI et al. Identification and Functional Analysis of Single Nucleotide Polymorphism in the Tandem Repeat Sequence of Thumidylate Synthase Gene. Cancer Research. 15 September 2003, Vol. 63, pages 6004-6007, see entire document.	1-10
X, P	MANDOLA et al. A Novel Single Nucleotide Polymorphism within the 5' Repeat Polymorphism of the Thymidylate Synthase Gene Abolishes USF-1 Binding and Alters	1-10
— A	Transcriptional Activity. Cancer Research. 01 June 2003, Vol. 62, pages 2898-2904, see entire document.	11-20
Y	TRINH et al. Thymidylate synthase: A novel genetic determinant of plasma homocysteine and folate levels. Human Genetics. July 2002, Volume 111, pages 299-302, see entire document.	11-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	IACOPETTA et al. A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. British Journal of Cancer. 2000, Volume 85, No. 6, pages 827-830, see entire document.	11-20
Y	ULRICH et al. Searching Expressed Sequence Tag Databases: Discovery and Confirmation of a Common Polymorphism in the Thymidylate Synthase Gene. Eancer Epidemology, Biomarkers & Prevention. December 2000, Vol. 9, pages 1381-1385, see entire document.	11-20
X	ISHIDA et al. Association of the Thymidylate Synthase Gene Polymorphism with its mRNA and Protein Expression and with Prognosis in Gastric Cancer. Anticancer Research. September 2002, Vol. 22, pages 2805-2810, see entire document.	11-20
X	CHEN et al. A novel polymorphis in the thymidylate synthase gene promoter influences plasma folate level and may modify the risk of colorectal cancer in a prospective study. Proceedings of the American Association for Cancer Research. March 2002, Vol. 43, page 659, Abstract #3272.	11-20
X --- Y	MARSH et al. They thymidylate Synthase enhancer region polymorphism in colorectal cancer. Proceedings of the American Association for Cancer Research. March 1998, Volume 39, pate 434, Abstract # 2955	11-16 --- 17-20
A	TSUJI et al. Polymorphism in the Thymidylate Synthase Promoter Enhancer Region is Not an Efficacious Marker for Tumor Sensitivity to 5-Fluorouracil-based Oral Adjuvant Chemotherapy in Colorectal Cancer. Clincial Cancer Research. 01 September 2003, Vol. 9, pages 3700-3704.	11-20
A	MARSH et al. Ethnic Variation in the Thymidylate Synthase Enhancer Region Polymorphism among Caucasian and Asian Populations. Genomics. 1999, Vol. 58, pages 310-312.	1-20

INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group 1, claims 1-10, drawn to isolated nucleic acid molecules, probes and kits.

Group 2, claims 11-20, drawn to methods for determining whether an individual has a heightened predisposition to cancer or cardiovascular disease.

There is no special technical feature which joins groups I and II, as the methods of claim 11 do not recite or require the products of claim 1 or invention 1. Even if they were to recite or require the products of the main invention, the main invention does not represent an advance in view of the prior art. Lou *et al.* (GenBank AF279906) teach an isolated nucleic acid comprising SEQ ID NO: 1, wherein G is replaced by C at nucleotide 12 (see nucleotides 132-159 of Lou *et al.*). Furthermore, with regard to claim 3, Dean *et al.* (US6087489) teach a single-stranded nucleic acid probe that hybridizes to the isolated nucleic acid molecule of claim 1. Specifically, SEQ ID NO: 16 taught by Dean *et al.* is a 20mer nucleic acid probe which is complementary to nucleotides 7-26 of instant SEQ ID NO: 1, wherein G is replaced by C at nucleotide 12. PCT Rule 13.2 states "The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes ***over the prior art*** (emphasis added)." Since the main invention was known at the time of filing, there is a lack of unity of invention between group 1 and group 2.